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Proteoglycans from Human Articular Cartilage: The Effect of Joint Location on the Structure[1], [2])

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Dedicated to Prof. Dr. Axel Delbrück on the occasion of his 60th birthday

Summary: Proteoglycan monomers from the articular cartilages of the knee, hip and shoulder of 3 subjects (21, 26 and 45 years old) were isolated and analysed. The proteoglycan monomers from the high weight-bearing knee and hip joints were smaller than those from the low weight-bearing shoulder joints and both had a lower chondroitin sulphate content. The proteoglycan monomers from knee joint cartilage had the lowest intra-individual chondroitin-4-sulphate content in each case. Hyaluronate binding capacity was not found to be dependent on joint location.

Der Einfluß der Gelenklokalisation auf die Struktur menschlicher Knorpelproteoglykane

Zusammenfassung: Proteoglykanmonomere aus den Gelenkknorpeln der Knie, Hüfte und Schulter wurden bei 3 Probanden (21, 25 und 45 Jahre alt) isoliert und analysiert. Die Proteoglykanmonomere der hoch gewichtsbelasteten Knie- und Hüftgelenke erwiesen sich als kleiner als die der weniger gewichtsbelasteten Schultergelenke und hatten jeweils einen niedrigeren Chondroitinsulfatgehalt. Die Proteoglykanmonomere der Kniegelenkknorpel zeigten intraindividuell jeweils den niedrigsten Anteil Chondroitin-4-sulfat. Eine Abhängigkeit der Hyaluronatbindungsfähigkeit von der Gelenklokalisation wurde nicht festgestellt.

Introduction

Since the first description by *Hascall & Sajdera* (1) of a method for isolating intact proteoglycans from cartilage tissue, the structure of these connective tissue components has been investigated continuously. Varying structures can be assigned to different tissues (see *Hascall & Kimura* (2) for a review). The proteoglycans of hyaline articular cartilage contain a central core protein. Roughly two-thirds of this protein are occupied by glycosaminoglycan side-chains, with one-third being assigned to the chrondroitin sulphate-rich region and one-third to the keratan sulphate-rich region. The remaining one-third is de-

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[2]) This work contains parts of the doctoral thesis of *G. Pallasch.*

void of glycosaminoglycans and binds to hyaluronic acid. Systematic structural changes have been described in connection with ageing processes and degenerative disorders (3).

The extent to which functional biomechanical stresses on the joints might contribute to these changes remains uncertain. In vitro studies have demonstrated that mechanical stresses affect the biosynthesis and breakdown of cartilage proteoglycans (4, 5). In in vivo studies, proteoglycans with differing structures have been detected in rabbit knee joint cartilage subjected to varying stress: the proteoglycans of the higher weight-bearing joints contained a greater glycosamine portion, indicating a larger keratan sulphate region (6). In contrast, comparative analysis of proteoglycans from high weight-bearing human knee joint cartilage and low weight-bearing human shoulder joint cartilage failed to disclose any intra-individual structural differences (7). However, apart from one 34-year-old, all the subjects studied were 15 years old or less.

It was of interest to establish whether this finding also applies for proteoglycans from adult human articular cartilage, or whether proteoglycan structure is demonstrably affected by weight-bearing differences. Proteoglycans from the articular cartilage of shoulder, hip and knee of adult subjects were therefore analysed and compared on an intra-individual basis.

Materials and Methods

Cartilage

Human articular cartilage was obtained from the shoulder, hip and knee of 3 subjects (21, 26 and 45 years old) within 24 h post mortem. Left and right joints were investigated in parallel, except in the case of the 45-year-old subject where the right joints only were analysed. There was no clinical or macroscopic evidence of joint disease or of damage due to trauma. Histological examination after staining with safranin O and haematoxylin-eosin (8) revealed normal cartilage structure without any indication of pathological change.

Chemicals

The following materials were purchased from commercial sources: papain from Boehringer Mannheim (Mannheim, FRG), hyaluronate lyase (EC 4.2.99.1) from E. Merck (Darmstadt, FRG), chrondroitinase AC (EC 4.2.2.5), chondroitinase ABC (EC 4.2.2.4), hyaluronic acid (human umbilical cord), 6-aminohexanoic acid and soya bean trypsin inhibitor from the Sigma Chemical Company (Munich, FRG), standard disaccharides (2-acetamido-2-deoxy-3-O-(β-*D*-gluco-4-enepyranosyluronic acid)-*D*-galactose (Δ DiOS), 2-acetamido-2-deoxy-3-O-(β-*D*-gluco-4-enepyranosyluronic acid)-4-O-sulpho-*D*-galactose (Δ Di4S), 2-acetamido-2-deoxy-3-O-(β-*D*-gluco-4-enepyranosyluronic acid)-6-O-sulpho-*D*-galactose (Δ Di6S)) from Miles Biochemicals (Frankfurt, FRG) and Sephacryl S 500 from Pharmacia (Freiburg, FRG). All other chemicals (E. Merck, Darmstadt, FRG) were of analytical grade.

Extraction of proteoglycans

The cartilages were rinsed with a sodium chloride solution (155 mmol/l), sliced with a scalpel as small as possible and extracted with 4 mol/l guanidinium hydrochloride/0.05 mol/l sodium acetate, pH 6.0, in the presence of 0.1 mol/l 6-amino-hexanoic acid, 0.01 mol/l EDTA, 5 mmol/l benzamidine hydrochloride and 10 mg/l soya bean trypsin inhibitor (9). The extraction procedures were performed twice (24 h, 4 °C, 10 ml/0.5 g wet weight) and the extracts were pooled and concentrated by ultrafiltration (4 °C) to a volume of 25 ml/sample (Immersible CX 10, Millipore, Neu-Isenburg, FRG).

Ultracentrifugation

The proteoglycan monomers were isolated by dissociative density gradient centrifugation in caesium chloride (starting density: 1.53 kg/l, 371 000 *g*, 5 h, 4 °C, Beckman vertical tube rotor VTI 65, Beckman L 8 centrifuge). Each gradient was divided into 4 fractions each consisting of 1.2 ml. The two high-density bottom fractions were pooled and recentrifuged twice under the same conditions. Density (by gravimetry), uronic acid (10) and protein (11) were analysed in each fraction after dialysis against doubledistilled water (3 × 24 h).

The bottom fractions were used for further investigation after dialysis against doubledistilled water (3 × 24 h).

Gel chromatography

Proteoglycan samples (1 ml) from the bottom fractions were applied to a column (95 × 1 cm) of Sephacryl S 500 and eluted with 0.1 mol/l sodium acetate 0.1 mol/l sodium chloride, pH 6.8, at 9 ml/h and 4 °C. Uronic acid (10) and protein (11) were determined in each fraction (3 ml). The void volume and total volume of the column were determined using hyaluronic acid and glucuronolactone respectively. Hydrodynamic proteoglycan size was estimated by calculating k_{av} at the peak maximum (12).

Proteoglycan monomers were mixed with hyaluronic acid (20% based on uronic acid content) in order to estimate their ability to form aggregates with exogenous hyaluronic acid. The extent of the interaction was determined by gel chromatography on Sephacryl S 500 as described above. The proteoglycans bound to hyaluronic acid were eluted in the void volume. The hyaluronic acid-bound proteoglycan portion was calculated by subtracting the uronic acid content of the added hyaluronic acid from the overall uronic acid content of the void volume.

Isolation of glycosaminoglycans

In order to determine the glycosaminoglycan distribution patterns in the residue, in the overall extract and in the proteoglycan monomers, glycosaminoglycans were isolated as described previously (13). Briefly, the samples were digested with papain (50 mmol/l phosphate buffer, pH 6.0, 20 mmol/l EDTA, 5 mmol/l cysteine, 1500 U/l papain, 16 h, 60 °C) and the nonglycosaminoglycan components were precipitated with concentrated HCl (pH 1.5, 16 h, 4 °C). The precipitate was discarded and the supernatant was neutralized with 3 mol/l NaOH. After dialysis against doubledistilled water (2 × 24 h), freezedrying, β-elimination in 0.3 mol/l NaOH (4 h, 4 °C), neutralization with 3 mol/l HCl, dialysis against doubledistilled water (2 × 24 h) and precipitation with ethanol (5-fold volume), the resultant glycosaminoglycans were dissolved in doubledistilled water and analysed by the carbazol assay method (10). The degree of extraction was calculated from the sum of the uronic acid in the extracts and residues.

Determination of glycosaminoglycan components

The procedure used for the analysis of glycosaminoglycan distribution patterns was based on the digestion of the polymers by hyaluronate lyase (13), followed by chondroitinase AC and ABC (14), as described elsewhere (15). Each enzymatic degradation step was followed by separation of the metabolites from the undigested glycosaminoglycans by precipitating the undigested glycosaminoglycans with a four fold volume of sodium acetate-saturated ethanol (16 h, 4 °C). The residue was washed twice with 1 ml ethanol (96%). The supernatants from the precipitation and the wash procedures were pooled, evaporated to dryness (37 °C) and resolved in 200 µl doubledistilled water. The determinations of the components were performed by high performance liquid chromatography of the respective metabolites, as described in a previous publication (16). Keratan sulphate was analysed by determining the hexosamine content (17) of the hydrolysed residue (2 mol/l HCl, 16 h, 104 °C) precipitated after degradation with chondroitinase ABC. Glycosaminoglycans corresponding to amounts of 0.5 to 1.0 µmol uronic acid were used for each assay.

Results

Uronic acid content and degree of extraction of articular cartilage

The uronic acid concentration in the articular cartilage samples ranged from 10.7 to 28.8 mmol/kg wet weight (tab. 1). The highest uronic acid concentrations by far were detected in the hip and knee joints of the 26-year-old subject. In this case, as with the other joints, there was a high measure of consistency between the concentrations in left and right joints. The articular cartilages of the 45-year-old subject had the lowest uronic acid content. There was no systematic variation in uronic acid content as a function of joint location.

Tab. 1. Analytical data on cartilage, residue and extract. Index number: Age of cartilage investigated.
HA = hyaluronate;
Ch = chondroitin;
CS6, CS4 = chondroitin sulphate 6 resp. 4;
DS6, DS4 = dermatan sulphate-like fractions;
KS = keratan sulphate.

Location of joint		Content of uronic acid (mmol/kg wet weight)	Degree of extraction (fraction of uronic acid)	Glycosaminoglycan distribution pattern												
				Residue							Extract					
				glycosaminoglycan component (mol %)							glycosaminoglycan component (mol%)					
				HA	Ch	CS6	CS4	DS6	DS4	KS	Ch	CS6	CS4	DS6	DS4	KS
$Shoulder_{21}$	right	15.7	0.48	5.8	4.5	62.6	6.6	0.7	2.9	18.2	2.6	61.7	5.8	3.4	7.0	19.3
	left	18.4	0.53	6.1	4.7	62.1	5.3	0.9	2.9	17.9	2.7	65.1	4.9	2.7	6.7	17.8
Hip_{21}	right	18.9	0.54	5.0	5.6	59.7	5.2	0.5	1.3	22.5	3.9	65.6	4.8	0.8	1.3	23.6
	left	16.7	0.54	5.6	5.5	60.0	5.0	0.4	1.2	22.4	3.5	66.7	4.9	1.2	1.5	22.1
$Knee_{21}$	right	15.3	0.51	2.8	7.1	59.1	2.7	2.3	4.4	21.4	2.9	66.3	3.2	1.5	3.6	22.5
	left	12.3	0.49	2.0	8.6	59.2	3.1	2.4	4.7	20.1	4.6	62.9	3.1	1.8	6.3	21.3
$Shoulder_{26}$	right	14.2	0.43	3.6	7.2	62.6	5.9	0.9	3.1	16.8	5.5	63.6	7.3	1.4	3.0	19.1
	left	15.9	0.44	5.4	5.7	62.3	7.2	0.3	1.7	17.3	5.1	63.4	6.4	1.3	3.5	19.4
Hip_{26}	right	23.2	0.67	6.9	7.0	58.1	4.0	0.5	1.1	22.5	4.9	65.7	5.3	0.8	1.0	22.2
	left	21.1	0.51	6.9	6.9	57.2	4.7	0.9	1.3	22.3	4.2	66.5	4.9	1.2	1.3	22.0
$Knee_{26}$	right	26.5	0.63	2.1	7.4	64.3	2.9	0.7	2.1	20.4	3.3	62.2	3.4	1.3	4.7	25.1
	left	28.8	0.62	2.2	7.7	62.7	2.9	1.0	2.0	21.6	3.8	63.0	3.4	1.6	6.2	22.0
$Shoulder_{45}$	right	10.7	0.50	1.0	15.2	47.7	5.5	0.5	1.2	28.7	3.6	54.0	5.2	2.3	3.8	31.0
Hip_{45}	right	15.4	0.54	1.8	12.5	47.4	3.9	0.9	0.3	33.2	4.7	54.3	4.5	0.4	0.7	35.4
$Knee_{45}$	right	12.0	0.58	1.3	8.6	52.5	2.8	0.7	0.7	33.2	4.5	55.2	2.3	1.0	2.3	34.7

The degree of extraction was calculated from the difference between the uronic acid content of the articular cartilages and the extracted uronic acids. In each case, an approximately identical degree of extraction was recorded for left and right joints (tab. 1). The only exceptions were the hip joints of the 26-year-old subject. The degree of extraction did not depend systematically on joint location, articular cartilage uronic acid content or the subject's age.

The glycosaminoglycan distribution pattern of proteoglycans from the residue and extract

After proteolysis, the glycosaminoglycans from the non-extractable proteoglycans of the residue are amenable to analysis. In order to establish whether the extracted proteoglycans differed from those in the residue, the glycosaminoglycan distribution patterns of the extraction residues were determined and compared with those of the extracts.

The principal components of the cartilage fractions were chondroitin-6-sulphate and keratan sulphate (tab. 1). Only the residue contained hyaluronic acid (which thus proved to be non-extractable under these conditions). The left and right sides in the 21-year-old and 26-year-old subjects were consistent for both fractions, given the range of the method. Apart from the non-extractable hyaluronic acid, another major difference was the higher chondroitin content in the residue, this being especially pronounced in the shoulder and hip joint cartilage of the 45-year-old subject. The differences were significant (paired t-test, $p < 0.001$).

In both cartilage fractions the keratan sulphate content of the glycosaminoglycans of the shoulder joints was invariably lowest. Similarly, the chondroitin-4-sulphate content displayed a systematic dependence on the location of the articular cartilage, being lowest in all knee joint cartilages. The hyaluronate content was also lowest in each case in the extraction residues of the knee joint cartilages from the 21-year-old and 26-year-old subjects. No such dependence was found in the 45-year-old subject. In this instance, however, the hyaluronate content in all joints was lower than in the younger subjects.

Isolation and analysis of proteoglycan monomers

The proteoglycan monomers were isolated from the total extract by density gradient centrifugation on a vertical tube rotor. Because of the smaller separation distance, the run time required to establish the density gradient is shorter with this rotor type than with fixed angle rotors (18). In the rotor used in the present study, the gradient was established within 5 hours (371 000 *g*, 65 000 min^{-1}). The proteoglycan monomers from the densest fraction of the third run were analysed with reference to size, hyaluronate binding capacity, uronic acid and protein content, and glycosaminoglycan distribution pattern. A fraction of about 0.8 of the extracted uronic acids was obtained after proteoglycan isolation.

Table 2 shows that the uronic acid/protein ratio did not depend systematically on joint location. Left- and right-sided results for the 21-year-old and 26-year-old subjects were highly consistent. Analysis of hydrodynamic molecular size by gel chromatography revealed that the proteoglycan monomers of the shoulder joint cartilage were largest in all three subjects. They had both the highest portion of molecules eluted with the void volume (fig. 1) and the lowest individual k_{av} values. The proteoglycan monomers from the hip and knee joint cartilages differed only slightly. No correlation was established between the uronic acid/protein ratio and k_{av}.

Tab. 2. Characteristic data on proteoglycan monomers: uronic acid/protein weight ratio, k_{av} calculated at peak maximum of gel chromatography, and the fraction of hyaluronate-binding proteoglycan monomers. The interaction with hyaluronate was quantified by determining the fraction of proteoglycan uronic acid coeluted with hyaluronate. n. d. = not determined.

Location of joint		Uronic acid / Protein	k_{av}	Proteoglycan/ hyaluronate interaction (fraction of proteoglycan)
$Shoulder_{21}$	right	3.2	0.23	1.0
	left	3.4	0.23	1.0
Hip_{21}	right	1.4	0.35	1.0
	left	1.8	0.35	1.0
$Knee_{21}$	right	1.8	0.39	0.9
	left	1.4	0.35	0.9
$Shoulder_{26}$	right	2.2	0.19	1.0
	left	2.3	0.23	n. d.
Hip_{26}	right	1.9	0.39	1.0
	left	2.1	0.35	n. d.
$Knee_{26}$	right	1.0	0.30	0.9
	left	1.1	0.35	n. d.
$Shoulder_{45}$	right	1.2	0.35	0.36
Hip_{45}	right	2.6	0.46	0.46
$Knee_{45}$	right	2.8	0.42	0.37

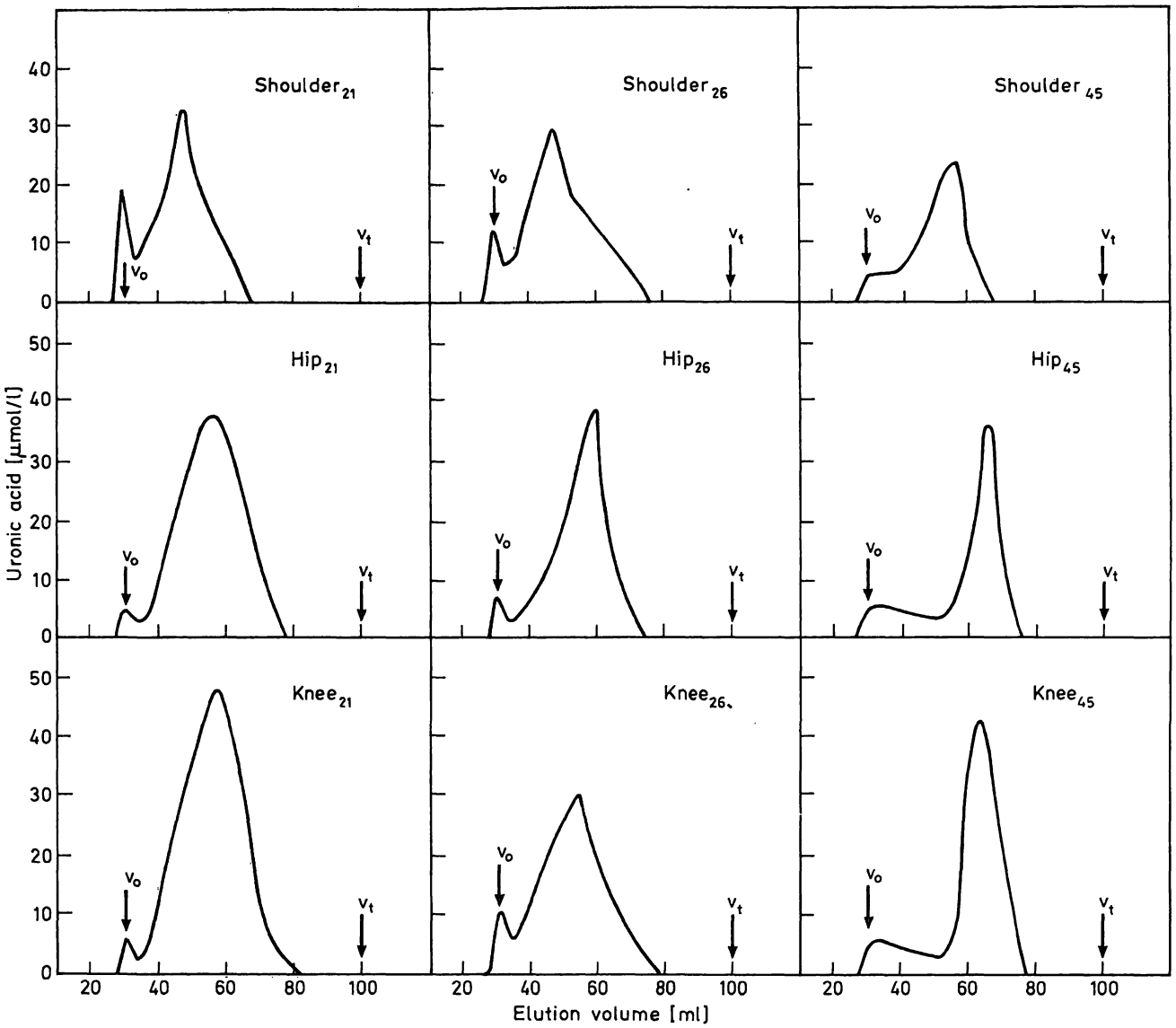

Fig. 1. Gel chromatography of proteoglycan monomers isolated from cartilage from the right-sided joints of the subjects. (Sephacryl S 500; 0.1 mol/l sodium acetate / 0.1 mol/l sodium chloride, pH 6.8; 9 ml/h.)

Proteoglycans form link-protein-stabilised complexes with hyaluronate in vivo (19—21). Complex formation in the absence of link protein in vitro occurs as a reversible equilibrium reaction (22, 23). Complex formation is temperature-dependent (23, 24). The experiments to determine the portion of complex-binding proteoglycan monomers were therefore conducted at constant temperature (4 °C) and under hyaluronate excess in order to force the equilibrium as far as possible towards the complex. Excess uronic acid here does not interfere with hyaluronate-proteoglycan complex formation (23). The analyses were performed by gel chromatography. The hyaluronate used was eluted with the void volume (fig. 2). The hyaluronate binding capacity was calculated as the percentage portion of the proteoglycan monomers coeluted with hyaluronate in the void volume (calculation based on uronic acid). The proteoglycan monomers of the shoulder and hip joints of the 21-year-old and 26-year-old subjects were characterised by 100% hyaluronate binding capacity.

Complex formation was observed in only 90% of the proteoglycan monomers from the knee joint cartilages of these two subjects. In the case of the 45-year-old subject, the hyaluronate binding capacity of the proteoglycan monomers was limited to about 40% (tab. 2, fig. 2); however, no systematic pattern dependent on joint location was detected here either.

To permit determination of the glycosaminoglycan distribution patterns, the proteoglycan monomers from left and right-sided joints from the 21-year-old and 26-year-old subjects were pooled so as to provide a sufficient quantity for a complete analysis. As in the corresponding analyses of the glycosaminoglycans in the residues and extracts, chrondroitin-6-sulphate

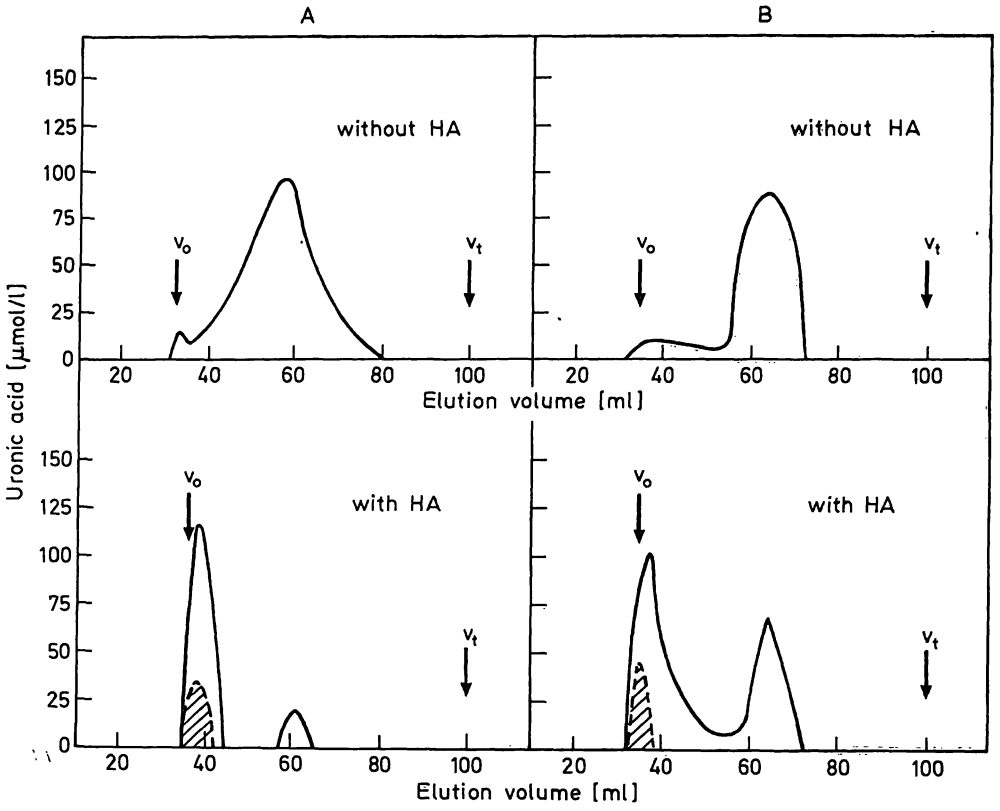

Fig. 2. Determination of hyaluronate-binding proteoglycans. A = proteoglycan monomers isolated from the knee joint cartilage of the 21-year-old subject. B = proteoglycan monomers isolated from the knee joint cartilage of the 45-year-old subject. Chromatography was performed with and without hyaluronate (HA) added. ▨ = hyaluronate alone. Conditions of chromatography: Sephacryl S 500; 0.1 mol/l sodium acetate / 0.1 mol/l sodium chloride, pH 6.8; 9 ml/h.

and keratan sulphate were found to be the principal constituents (tab. 3). As anticipated, hyaluronate was not detected in any sample. One notable systematic variation dependent on joint location was the fact that the keratan sulphate content of shoulder joint cartilage was lowest in each individual subject. As with the distribution patterns of the two cartilage fractions in table 1, the proteoglycan monomers of the knee joints had the lowest chondroitin-4-sulphate content in each case here (tab. 3). All other components failed to display any systematic variation as a function of joint location. Overall, in the case of the 45-year-old subject, the content of chondroitin-6-sulphate was lowest and the content of keratan sulphate was highest of all the subjects examined.

Discussion

Method

Human articular cartilage contains a heterogeneous proteoglycan population. Thus, systematic intraarticular structural changes have been described as a function of distance from the cartilage surface (25, 26). Given the uniform macroscopic and histological intactness of the cartilage samples in the present study, this heterogeneity was ignored since its effect on the analytical results was presumably identical for each sample. Extraction yields are affected to a considerable degree by the technique used for tissue comminution (26). The yields reported in the literature for adult human articular cartilage (50–60% of uronic acid) (7, 23) were achieved with the technique employed here. Since the cartilage discs were certainly 250 µm or more thick, it was not possible (see also *Bayliss* (26)) to extract hyaluronate because section thicknesses of the order of 100 µm are required in order to achieve this.

The uronic acid/keratan sulphate ratios in the residue and extract did not differ significantly (tab. 4, $p > 0.2$, paired t-test). There was therefore no selection of the proteoglycan monomers by extraction with respect to their glycosaminoglycan composition, such as reported by *Bayliss* et al. (26) for the articular cartilages of subjects approximately 75 years old. In our own experiments, significantly lower uronic acid/keratan sulphate ratios in the extracts have also only been detected in the articular cartilage of older subjects (62–74 years old) (27, 28). Consequently, an effect may be postulated which only occurs in subjects of more advanced age. In agreement with this theory, the structural differences in proteoglycans dependent on joint location in the subjects examined here were encountered both in the non-extractable and in the extracted fractions.

Tab. 3. Glycosaminoglycan distribution patterns of proteoglycan monomers. Data in mol% of total glycosaminoglycans. Proteoglycan monomers from the left and right joints from the 21-year-old and 26-year-old subjects were pooled.
Ch = chondroitin;
CS6, CS4 = chondroitin sulphate 6 or 4;
DS6, DS4 = dermatan sulphate-like fractions;
KS = keratan sulphate.

Location of joint	Glycosaminoglycan component (mol%)					
	Ch	CS6	CS4	DS6	DS4	KS
$Shoulder_{21}$	3.3	65.4	5.0	4.2	3.4	18.6
Hip_{21}	3.5	67.2	5.4	1.1	1.0	21.8
$Knee_{21}$	3.0	67.1	3.6	1.2	0.8	24.6
$Shoulder_{26}$	3.7	71.3	5.3	0.4	0.6	18.6
Hip_{26}	4.8	65.5	5.1	1.2	0.7	22.7
$Knee_{26}$	4.8	67.2	3.9	0.4	0.3	23.4
$Shoulder_{45}$	4.2	62.1	3.0	0.4	0.5	29.7
Hip_{45}	4.4	57.6	3.6	0.4	1.9	32.2
$Knee_{45}$	3.4	59.8	2.8	0.1	0.2	33.6

Tab. 4. Uronic acid/keratan sulphate and chondroitin-6-sulphate/chondroitin-4-sulphate molar ratios calculated from tables 1, 2 and 3. Samples from left and right joints were pooled for the analysis of proteoglycan monomers. Index number: Age of cartilage investigated.

Location of joint		Uronic acid/keratan sulphate molar ratio			Chondroitin-6-sulphate/chondroitin-4-sulphate molar ratio		
		Residue	Extract	Proteoglycan monomers	Residue	Extract	Proteoglycan monomers
$Shoulder_{21}$	left	4.2	4.1	4.3	9.5	10.6	13.1
	right	4.1	4.6		11.7	13.3	
Hip_{21}	left	3.2	3.2	3.5	11.5	13.7	12.4
	right	3.2	3.2		12.0	13.6	
$Knee_{21}$	left	3.5	3.4	3.1	21.9	20.7	18.6
	right	3.8	3.7		19.1	20.3	
$Shoulder_{26}$	left	4.6	4.2	4.3	10.6	8.7	13.5
	right	4.1	4.1		8.7	9.9	
Hip_{26}	left	3.2	3.5	3.4	14.5	12.4	12.9
	right	3.2	3.5		12.2	13.6	
$Knee_{26}$	left	3.8	3.0	3.2	22.2	18.3	17.2
	right	3.5	3.5		21.6	18.5	
$Shoulder_{45}$	right	2.4	2.2	2.4	8.7	10.4	20.7
Hip_{45}	right	2.0	1.8	2.1	12.2	12.1	16.0
$Knee_{45}$	right	2.0	1.9	1.8	18.8	24.0	21.4

In agreement with earlier investigations (13), determination of the glycosaminoglycan distribution patterns repeatedly disclosed a fraction which was not digestable by chondroitinase AC but was digestable by chondroitinase ABC. More extensive investigation revealed that these were tetra- and hexasaccharide fragments which were sulphated predominantly at the C_4 atom of the galactosamine and could not be digested by chondroitinase AC even when isolated (16). This fraction should represent cartilage dermatan sulphate (14). However, one has to consider that these oligosaccharides contain one α-β unsaturated carbonic acid at their non-reducing end, which has been formed from glucuronic acid. This part of the oligosaccharides formally should be derived from chondroitin sulphate. In contrast, it has been reported that dermatan sulphate is a hybrid molecule always containing glucuronic acid moieties as integral parts of the carbohydrate chains (29). Hitherto no additional data on the molecular structure of cartilage dermatan sulphate polysaccharide chain have been reported. Therefore, in view of the specifity of the enzymes, this fraction was assigned to "dermatan sulphate like".

The effect of joint location on the structure of cartilage proteoglycans

The results indicate that, in the subjects examined, intra-individual structural differences exist, which depend on joint location. Thus, the proteoglycan monomers from the high weight-bearing knee and hip joints were invariably smaller than those from the low weight-bearing shoulder joints. This finding may presumably be attributed to a smaller chondroitin sulphate-rich region since the uronic acid/keratan sulphate ratios were also always lower in the high weight-bearing joints (tab. 4). Furthermore, the two high weight-bearing joints (knee and hip) differed in all three cartilage fractions in terms of their chondroitin-6-sulphate/chondroitin-4-sulphate ratio: the highest ratio was always found in the knee joint (tab. 4). These results are not consistent with those of *Roughley* (7) who found no significant differences in the molecular size and structure of the proteoglycan monomers of shoulder joint cartilage compared with those of the knee. However, his subjects were very young (all were 0—15 years old, except one who was 34 years old). It may be that these differences only become apparent in adult subjects. This is especially probable if functional causes could be found for the variations.

A reduction in the size of the chondroitin sulphate-rich region with increasing age has been described for human cartilage proteoglycans (30, 32). This find-

ing has been attributed to reduced UDP-xylose: core protein xylosyl transferase activity with increasing age (33). The smaller chondroitin sulphate-rich region in the knee and hip joints could thus be ascribed to more rapid ageing due to higher weight-bearing. Since weight-bearing continues as the subject grows older, the differences in the size of the condroitin sulphate-rich regions of the proteoglycans of shoulder joint cartilage on the one hand and of knee and hip joint cartilage on the other should become increasingly pronounced.

The chondroitin-6-sulphate/chondroitin-4-sulphate ratio in human articular cartilage increases with age (31, 34). However, the chondroitin-6-sulphate/chondroitin-4-sulphate ratio of the high weight-bearing articular cartilages is not consistent with the picture of premature ageing. More rapid ageing of the hip and knee joint cartilage should result in a higher ratio of chondroitin sulphate isomers. In fact, this was only found for the knee joints of the 21-year-old and 26-year-old subjects; in contrast, the data for the shoulder and hip joints were consistent (tab. 4). If the process of ageing is induced by the biomechanical stress, these differences between the high and the low weight-bearing joints should increase with increasing age. However, in the 45-year-old subject, these differences were only as marked as in the younger subjects in the residue and the extract. The differences in the chondroitin-4-sulphate content of the articular cartilage as a function of joint location cannot therefore be interpreted as indicating more rapid ageing of the weight-bearing knee and hip joints.

Arthrosis-induced changes in the structure of cartilage proteoglycans are characterised by a reduction in molecular size as a result of damage to the hyaluronate binding region (3). This is possibly caused by proteolytic destruction (35, 36) and altered synthesis in the chondrocytes (37, 38). The results of the present study do not offer any clear evidence of corresponding changes in the high weight-bearing articular cartilages. Thus, the hydrodynamic molecular size of the proteoglycans was not paralleled by either a definite restriction of hyaluronate binding capacity or any systematic change in the uronic acid/protein ratio. Where pronounced biomechanical stress on the articular cartilages also produces arthrosis-like changes in proteoglycan structure, these only appear to develop in relatively elderly subjects.

Our results have shown that the proteoglycans of the knee and hip joint cartilages were smaller than those of the shoulder joint cartilages because of the reduced chondroitin sulphate-rich region. The structural variations are biomechanically induced. However, it is not yet clear, whether they are the result of functional strain or/and of stress by weight-bearing. The differences do not fit in with the pattern of either age-induced or arthrosis-induced changes. More extensive investigations in healthy articular cartilage are required to clarify this state of affairs, and appropriate studies are in progress in our laboratory.

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